

Genetic Characterization of Loggerhead Turtles (*Caretta caretta*)

Nesting in Volusia County

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## Introduction

The loggerhead turtle is the only marine turtle that nests primarily outside the tropics. Major nesting aggregates exist in warm temperate regions of the Atlantic, Indian, and west Pacific Oceans, but are absent from the central and east Pacific. Encalada et al. (1998) surveyed 10 nesting areas in the Atlantic and Mediterranean using the mitochondrial DNA (mtDNA) control region. Although sharing of haplotypes between geographic regions was observed, the evidence indicated that most nesting aggregates are genetically distinct populations. The distribution of mtDNA markers resulted in the delineation of (at least) 6 population genetic units in the Atlantic-Mediterranean region including three in the southeastern United States and correspond to nesting beaches from (1) NC, SC, GA and northeast FL: (2) south FL and (3) northwest FL. Encalada et al. (1998) hypothesized that the boundary for the Atlantic coast populations was at a well-known biogeographic break between Cape Canaveral and Amelia Island, Florida. Sampling of nesting locations in Volusia County and subsequent analysis of mtDNA sequences, may define where the genetic break (in terms of mtDNA lineages) occurs. This information has implications for biogeography and loggerhead natural history, but is motivated in large part by conservation concerns. Are the turtles nesting in this heavily developed area part of the large southern population, or affiliated with the declining population to the north?

## Materials and Methods

Samples from 49 nesting females were obtained by Volusia County Turtle Patrol volunteers from nests laid during a 10 day period: 17 June 98 through 26

June 98. Tissue from moribund hatchlings and embryos were placed in 15 ml SED buffer (Amos and Hoezel, 1991), and stored at room temperature. Whole DNA isolations from tissue samples were conducted with the phenol/chloroform method described by Hillis et al. (1996).

A 380 base-pair fragment located in the control region of the mitochondrial genome (mtDNA) was amplified with PCR methodology (Mullis & Faloona, 1987), using the primers TCR-5 and TCR-6 of Norman et al. (1994). Sequences from Volusia County were compared to loggerhead haplotypes from other nesting locations (Encalada et al., 1998). Chi square tests were applied to determine whether nesting areas on the north side of Ponce Inlet were significantly different from those on the south side of the inlet. The Volusia County nesting areas were subsequently combined and compared to previously defined management units.

### Results

49 samples were analyzed from Volusia County including 14 samples from the south side of Ponce Inlet that separates Daytona and New Smyrna beaches. Five different mtDNA sequences were observed corresponding to haplotypes A, B, C, J and M (Table 1).

Table 1: Distribution of haplotypes in loggerhead turtles nesting in northern and southern portions of Volusia County.

Haplotype	North of Jetty	South of Jetty
A	23	9
B	9	4
C	1	0
J	1	1
M	1	0

Of haplotypes observed in Volusia Co., A, B, and C are known to occur elsewhere in the southeast U.S. Haplotype M had not been found in previously surveyed nesting areas (Table 2). In addition, haplotype J, which was formerly believed to be endemic to Yucatan, Mexico, was also observed in Volusia County.

Table 2. Haplotype distribution among the management units in the southeastern U.S. adapted from Encalada et al. (1998).

Haplotype	Volusia Co	NWFL	SFL	NEFL-NC
A	32	34	22	104
B	13	4	24	1
C	1	2	2	0
E	0	0	1	0
G	0	2	1	0
J	2	0	0	0
M	1	0	0	0

Chi square tests indicated that the mtDNA haplotype frequencies of turtles nesting on either side of the inlet are not significantly different from each; therefore these nesting localities are not genetically distinct. These samples were combined for subsequent analysis of a comprehensive “Volusia County” population. The Volusia County population is significantly different ( $\chi^2=36.92$ ,  $p=0$ ) from the northeast FL-NC management unit. The haplotype frequencies of Volusia County nesting turtles are also significantly different from the south

Florida and northwest Florida management units, but the levels of significance are marginal ( $X=10.44$ ,  $p=0.042$  and  $X=9.67$ ,  $p=0.043$  respectively).

### Discussion

One of the exciting results of this study was the identification of haplotype M in Volusia County. This haplotype had previously been observed only on juvenile foraging grounds around the east Atlantic islands of the Azores and the Madeiras (Bolten et al., 1998). The possible origin of these turtles was unknown, but now at least some of these juveniles can be tentatively assigned to the Volusia County nesting cohort. The Volusia County nesting cohort may contribute at detectable levels to the pelagic juvenile habitats of the North Atlantic.

The Volusia County population of nesting loggerhead turtles is distinct in terms of mtDNA haplotype frequencies from the previously defined populations in northeast FL-NC and southern Florida. The split between Volusia and the northeastern populations is striking: a high number and diversity of haplotypes present on Volusia's beaches contrasted with low diversity in the northeast FL-NC management unit. The genetic differences from the southern populations are subtle. There is a statistically significant shift in the frequencies of haplotypes A and B (the two most common haplotypes in Florida) between Volusia and southern populations. In examining the rarer haplotypes, two (J and M) present in Volusia County have not been found in south Florida, while two (low frequency) haplotypes (E and G) found in south Florida are not present in the Volusia County sample of nesting females.

Our interpretation of this genetic data is that the Volusia population comprises a distinct management unit. For Volusia County to be considered a distinct management unit, the haplotypes that occur on Volusia's beaches should be present in frequencies that are statistically significant from the haplotype frequencies of adjacent beaches. Furthermore, presence of haplotypes in Volusia County that have not been found in the northern and southern management units would strengthen the interpretation of Volusia comprising a distinct management unit. Clearly there are haplotypes which occur in Volusia County that have not been found on the beaches of the two adjacent management units and vice versa.

Encalada et al. (1998) hypothesized that the genetic break in the nesting populations on the Atlantic coast is somewhere between Cape Canaveral and Amelia Island, Florida. This study shows that a population genetic discontinuity does occur in this area, with a sharp distinction between the northern and Volusia populations and a more subtle distinction between the Volusia and southern populations. Frequencies of haplotypes shared among Volusia and the neighboring beaches are significantly different.

We initiated this study to determine whether the Volusia County nesting cohort is affiliated with the large nesting population to the south or the smaller, declining nesting population to the north. The unexpected result is that we cannot assign the Volusia County nesting turtles to either population. When formulating management policy for threatened and endangered species the most conservative interpretation should represent the best interest for the species (see

Dizon). In this case, our provisional interpretation is that Volusia County represents an independent management unit.

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